Topics and Techniques for Forensic DNA Analysis

STR and Molecular Biology Artifacts

Houston DNA
Training Workshop

Houston, TX
April 3-4, 2007

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Advantages for STR Markers

- Small product sizes are generally compatible with degraded DNA and PCR enables recovery of information from small amounts of material
- Multiplex amplification with fluorescence detection enables high power of discrimination in a single test
- Commercially available in an easy to use kit format
- Uniform set of core STR loci provide capability for national and international sharing of criminal DNA profiles

Types of STR Repeat Units

Requires size based DNA separation to resolve different alleles from one another

- **Di**nucleotide (CA)(CA)(CA)(CA)
- **Tri**nucleotide (GCC)(GCC)(GCC)
- **Tetra**nucleotide (AATG)(AATG)(AATG)
- **Penta**nucleotide (AGAAA)(AGAAA)
- **Hexa**nucleotide (AGTACA)(AGTACA)

Short tandem repeat (STR) = microsatellite = simple sequence repeat (SSR)

Categories for STR Markers

Category	Example Repeat Structure	13 CODIS Loci
Simple repeats – contain units of identical length and sequence	(GATA)(GATA)(GATA)	TPOX, CSF1PO, D5S818, D13S317, D16S539
Simple repeats with non-consensus alleles (e.g., TH01 9.3)	(GATA)(GAT-)(GATA)	TH01, D18S51, D7S820
Compound repeats – comprise two or more adjacent simple repeats	(GATA)(GATA)(GACA)	VWA, FGA, D3S1358, D8S1179
Complex repeats – contain several repeat blocks of variable unit length	(GATA)(GACA)(CA)(CATA)	D21S11

These categories were first described by **Urquhart *et al.* (1994) *Int. J. Legal Med.* 107:13-20**

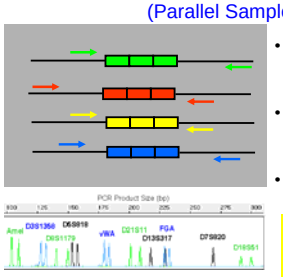
How many STRs in the human genome?

- The efforts of the Human Genome Project have increased knowledge regarding the human genome, and hence there are many more STR loci available now than there were 10 years ago when the 13 CODIS core loci were selected.
- **More than 20,000 tetranucleotide STR loci have been characterized in the human genome** (Collins et al. An exhaustive DNA micro-satellite map of the human genome using high performance computing. Genomics 2003;82:10-19)
- There may be more than a million STR loci present depending on how they are counted (Ellegren H. Microsatellites: simple sequences with complex evolution. Nature Rev Genet 2004;5:435-445).
- STR sequences account for approximately 3% of the total human genome (Lander et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860-921).

Butler, J.M. (2006) Genetics and genomics of core STR loci used in human identity testing. *J. Forensic Sci.* 51(2): 253-265.

Multiplex PCR

(Parallel Sample Processing)

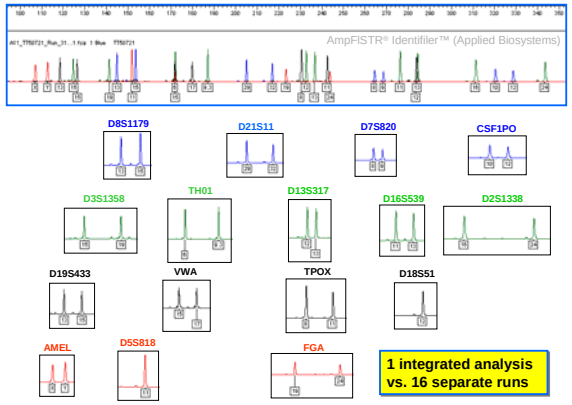


- **Compatible primers are the key to successful multiplex PCR**
- **STR kits are commercially available**
- **15 or more STR loci can be simultaneously amplified**

Challenges to Multiplexing
- primer design to find compatible primers (no program exists)
- reaction optimization is highly empirical often taking months

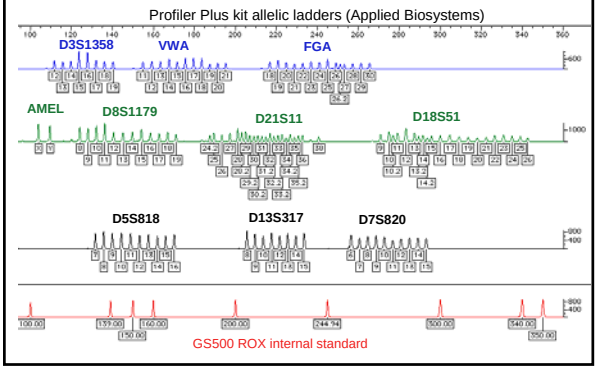
Advantages of Multiplex PCR
- –Increases information obtained per unit time (increases power of discrimination)
- –Reduces labor to obtain results
- –Reduces template required (smaller sample consumed)

Information is tied together with multiplex PCR and data analysis



1 integrated analysis vs. 16 separate runs

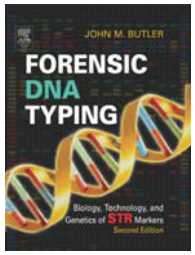
Companies Supply **Allelic Ladders** in STR Kits to **Aid Interlaboratory Consistency**



Biological "Artifacts" of STR Markers

- Stutter Products
- Non-template nucleotide addition
- Microvariants
- Tri-allelic patterns
- Null alleles
- Mutations

Chapter 6 covers these topics in detail



Stutter Products

- Peaks that show up primarily one repeat less than the true allele as a result of strand slippage during DNA synthesis
- Stutter is less pronounced with larger repeat unit sizes (dinucleotides > tri- > tetra- > penta-)
- Longer repeat regions generate more stutter
- Each successive stutter product is less intense (allele > repeat-1 > repeat-2)
- Stutter peaks make mixture analysis more difficult

STR Alleles with Stutter Products

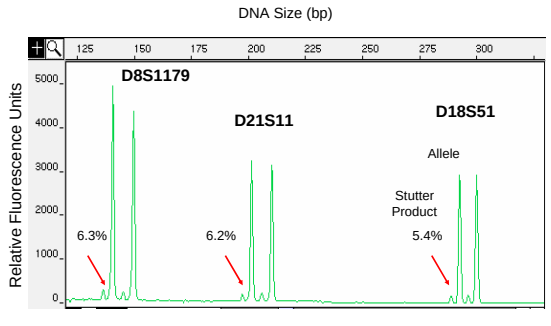
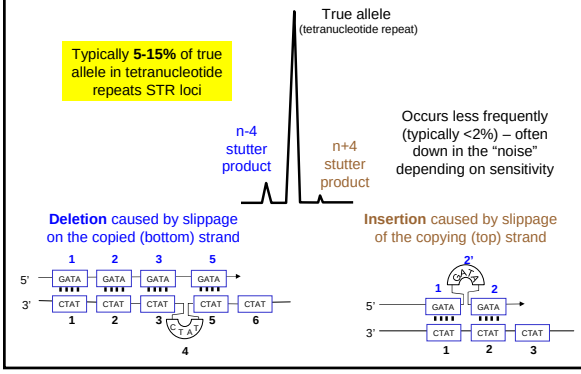


Figure 6.1, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

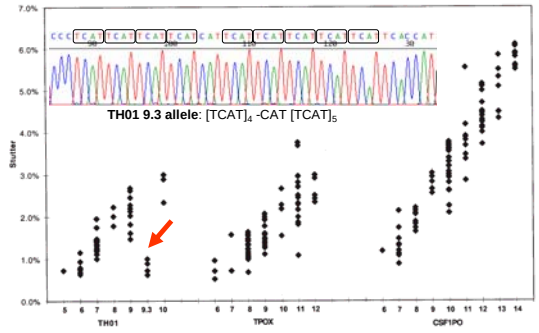
Stutter Product Formation

Repeat unit bulges out when strand breathing occurs during replication



Measured Stutter Percentages

Variable by Allele Length and Composition

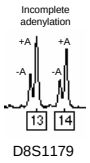


Holt CL, Buoncristiani M, Wallin JM, Nguyen T, Lazaruk KD, Walsh PS. TWGDAM validation of AmpFlSTR PCR amplification kits for forensic DNA casework. *J Forensic Sci* 2002; 47(1): 66-96.

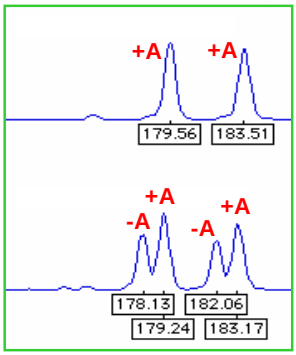
Non-Template Addition

- Taq polymerase will often add an extra nucleotide to the end of a PCR product; most often an "A" (termed "adenylation")
- **Dependent on 5'-end of the reverse primer; a "G" can be put at the end of a primer to promote non-template addition**
- Can be enhanced with extension soak at the end of the PCR cycle (e.g., 15-45 min @ 60 or 72 ºC) – to give polymerase more time
- Excess amounts of DNA template in the PCR reaction can result in incomplete adenylation (not enough polymerase to go around)

Best if there is NOT a mixture of "+/- A" peaks
(desirable to have full adenylation to avoid split peaks)



Impact of the 5' Nucleotide on Non-Template Addition



5'-ACAAG…

Last Base for Primer Opposite Dye Label

(PCR conditions are the same for these two samples)

5'-CCAAG…

Promega includes an ATT sequence on the 5'-end of many of their unlabeled PP16 primers to promote adenylation

see Krenke *et al.* (2002) *J. Forensic Sci.* 47(4): 773-785
http://www.cstl.nist.gov/biotech/strbase/PP16primers.htm

Higher Levels of DNA Lead to Incomplete Adenylation

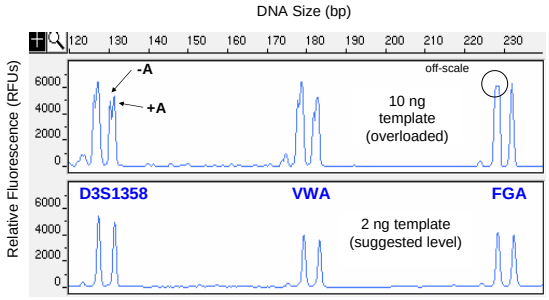


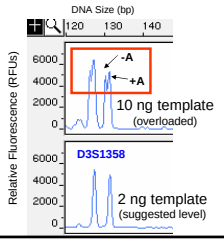
Figure 6.5, J.M. Butler (2005) *Forensic DNA Typing*, 2nd Edition © 2005 Elsevier Academic Press

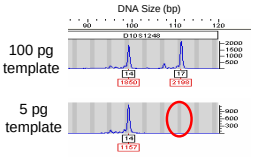
Impact of DNA Amount into PCR

Reason that DNA Quantitation is Important Prior to Multiplex Amplification

Generally 0.5 – 2.0 ng DNA template is best for STR kits

- Too much DNA
  - Off-scale peaks
  - Split peaks (+/-A)
  - Locus-to-locus imbalance
- Too little DNA
  - Heterozygote peak imbalance
  - Allele drop-out
  - Locus-to-locus imbalance

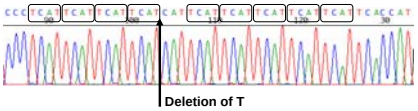




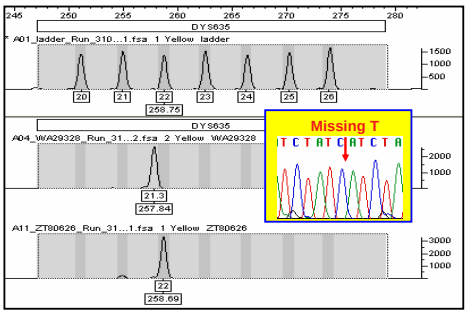
Stochastic effect when amplifying low levels of DNA produces allele dropout

Microvariant "Off-Ladder" Alleles

- Defined as alleles that are not exact multiples of the basic repeat motif or sequence variants of the repeat motif or both
- Alleles with partial repeat units are designated by the number of full repeats and then a decimal point followed by the number of bases in the partial repeat (Bar *et al. Int. J. Legal Med.* 1994, 107:159-160)
- Example: **TH01 9.3 allele**: $[TCAT]_4$ -CAT $[TCAT]_5$



An Example of an "Off-Ladder" Microvariant at the Yfiler Locus DYS635



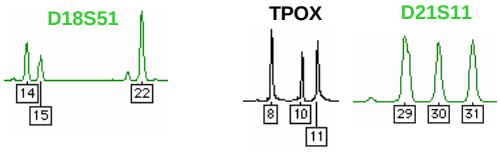
Allele 22 bin
258.75 +/- 0.5 = 258.25 to 259.25

Allele 21.3
257.84
(-0.41 from bin)

[TCTA]$_4$(TGTA)$_2$[TCTA]$_2$(TGTA)$_2$[TCTA]$_2$(TGTA)$_2$ [TCTA]$_5$ TC-A [TCTA]$_2$

Three-Peak Patterns

Clayton *et al.* (2004) A genetic basis for anomalous band patterns encountered during DNA STR profiling. *J Forensic Sci.* 49(6):1207-1214



"Type 1"

Sum of heights of two of the peaks is equal to the third

Most common in D18S51 and .....

"Type 2"

Balanced peak heights

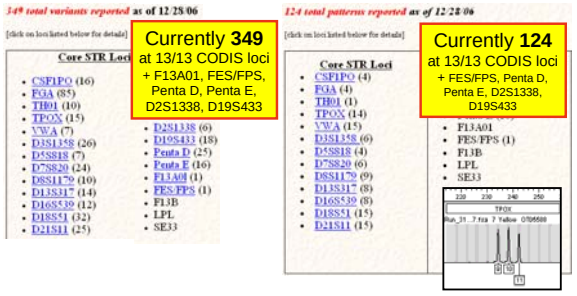
Most common in TPOX and D21S11

Variant Alleles Cataloged in STRBase

http://www.cstl.nist.gov/biotech/strbase/var_tab.htm

Off-Ladder Alleles

Tri-Allelic Patterns



Currently **349** at 13/13 CODIS loci + F13A01, FES/FPS, Penta D, Penta E, D2S1338, D19S433

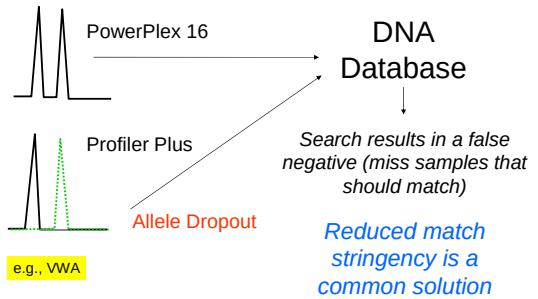
Currently **124** at 13/13 CODIS loci + FES/FPS, Penta D, Penta E, D2S1338, D19S433

Null Alleles

- Allele is present in the DNA sample but fails to be amplified due to a **nucleotide change in a primer binding site**
- Allele dropout is a problem because a heterozygous sample appears falsely as a homozygote
- Two PCR primer sets can yield different results on samples originating from the same source
- This phenomenon impacts DNA databases
- Large concordance studies are typically performed prior to use of new STR kits

For more information, see J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, pp. 133-138

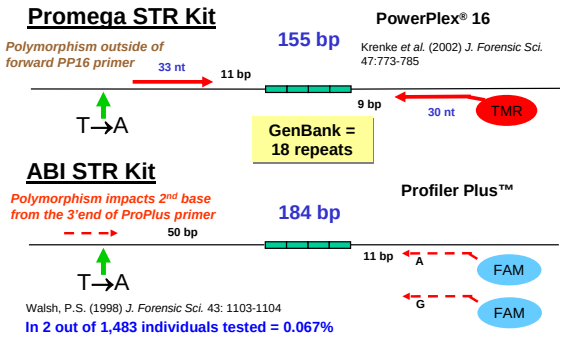
Concordance between STR primer sets is important for DNA databases



Search results in a false negative (miss samples that should match)

Reduced match stringency is a common solution

vWA Primer Position Comparisons

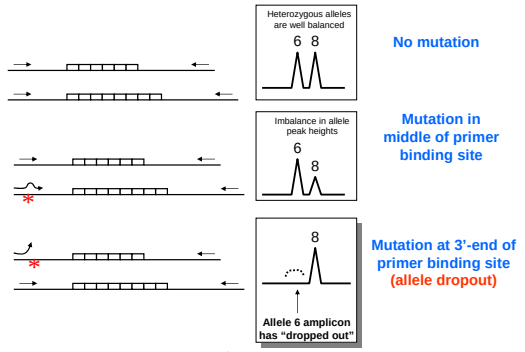


Walsh, P.S. (1998) *J. Forensic Sci.* 43: 1103-1104

In 2 out of 1,483 individuals tested = 0.067%

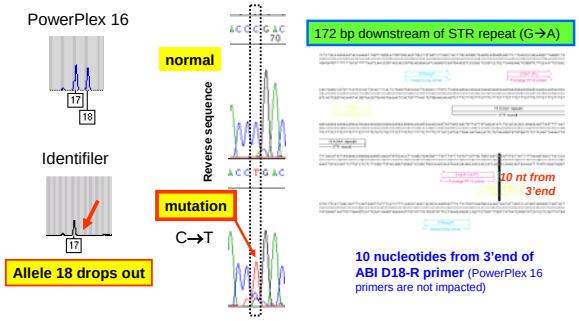
Lazaruk *et al.* (2001) *Forensic Sci Int.* 119:1-10

Impact of DNA Sequence Variation in the PCR Primer Binding Site



Butler, J.M. (2005) *Forensic DNA Typing, 2nd Edition*, Figure 6.9, ©Elsevier Academic Press

D18S51 Null Allele from Kuwait Samples with ABI Primers



Clayton et al. (2004) **Primer binding site mutations affecting the typing of STR loci contained within the AMPFISTR SGM Plus kit.** *Forensic Sci Int.* 139(2-3): 255-259

Apparent Null Alleles Observed During Concordance Studies

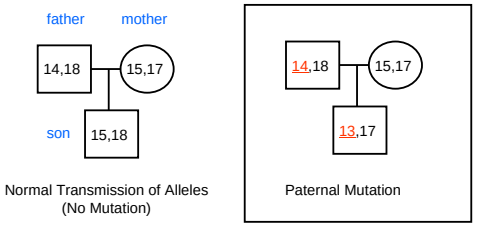
10/13 CODIS loci affected so far

New Section of STRBase (launched to track MiniFiler discordance and allele dropout frequency): http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm

Locus	STR Kits/Assays	Results	Reference
D13S317	Identifiler vs miniplexes	Shift of alleles 10 and 11 due to deletion outside of **miniplex assay**	Butler et al. (2003), Drabek et al. (2004)
D16S539	PP1.1 vs PP16 vs COfiler	Loss of alleles with **PP1.1**; fine with PP16 and COfiler	Nelson et al. (2002)
D8S1179	PP16 vs ProPlus	Loss of alleles 15, 16, 17, and 18 with **ProPlus**; fine with PP16	Budowle et al. (2001)
FGA	PP16 vs ProPlus	Loss of allele 22 with **ProPlus**; fine with PP16	Budowle and Sprecher (2001)
D18S51	SGM vs SGM Plus	Loss of alleles 17, 18, 19, and 20 with **SGM Plus**; fine with SGM	Clayton et al. (2004)
CSF1PO	PP16 vs COfiler	Loss of allele 14 with **COfiler**; fine with PP16	Budowle et al. (2001)
TH01	PP16 vs COfiler	Loss of allele 9 with **COfiler**; fine with PP16	Budowle et al. (2001)
D21S11	PP16 vs ProPlus	Loss of allele 32.2 with **PP16**; fine with ProPlus	Budowle et al. (2001)

From Table 6.2 in J.M. Butler (2005) *Forensic DNA Typing, 2nd Edition*, p. 136

Mutation Observed in Family Trio



Butler, J.M. (2001) *Forensic DNA Typing*, Figure 6.9, ©Academic Press

STR Measured Mutation Rates

http://www.cstl.nist.gov/biotech/strbase/mutation.htm

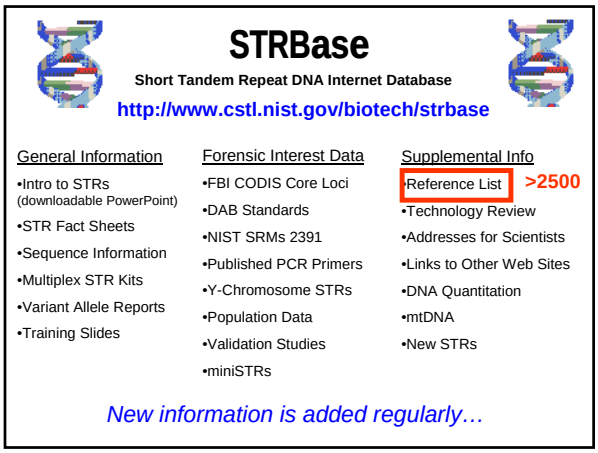
	STR Locus	Maternal Meioses (%)	Paternal Meioses (%)	Either Parent	Total Mutations	Rate
13 CODIS core loci	**CSF1PO**	70/179,353 (0.04)	727/504,342 (0.14)	303	1,100/683,695	**0.16%**
	FGA	134/238,378 (0.06)	1,481/473,924 (0.31)	495	2,110/712,302	**0.30%**
	TH01	23/189,478 (0.01)	29/346,518 (0.008)	23	75/535,996	**0.01%**
	TPOX	16/299,186 (0.005)	43/328,067 (0.01)	24	83/627,253	**0.01%**
	VWA	133/400,560 (0.03)	907/646,851 (0.14)	628	1,668/1,047,411	**0.16%**
	D3S1358	37/244,484 (0.02)	429/336,208 (0.13)	266	732/580,692	**0.13%**
	D5S818	84/316,102 (0.03)	537/468,366 (0.11)	303	924/784,468	**0.12%**
	D7S820	43/334,886 (0.01)	550/461,457 (0.12)	218	811/796,343	**0.10%**
	D8S1179	54/237,235 (0.02)	396/264,350 (0.15)	225	675/501,585	**0.13%**
	D13S317	142/348,395 (0.04)	608/435,530 (0.14)	402	1,152/783,925	**0.15%**
	D16S539	77/300,742 (0.03)	350/317,146 (0.11)	256	683/617,888	**0.11%**
	D18S51	83/130,206 (0.06)	623/278,098 (0.22)	330	1,036/408,304	**0.25%**
	D21S11	284/258,795 (0.11)	454/306,198 (0.15)	423	1,161/564,993	**0.21%**
	Penta D	12/18,701 (0.06)	10/15,088 (0.07)	21	43/33,789	**0.13%**
	Penta E	22/39,121 (0.06)	58/44,152 (0.13)	55	135/83,273	**0.16%**
	D2S1338	2/25,271 (0.008)	61/81,960 (0.07)	31	94/107,231	**0.09%**
	D19S433	22/28,027 (0.08)	16/38,983 (0.04)	37	75/67,010	**0.11%**
	F13A01	1/10,474 (0.01)	37/65,347 (0.06)	3	41/75,821	**0.05%**
	FES/FPS	3/18,918 (0.02)	79/149,028 (0.05)	None reported	82/167,946	**0.05%**
	F13B	2/13,157 (0.02)	8/27,183 (0.03)	1	11/40,340	**0.03%**
	LPL	0/8,821 (<0.01)	9/16,943 (0.05)	4	13/25,764	**0.05%**
	SE33 (ACTBP2)	0/330 (<0.30)	330/51,610 (0.64)	None reported	330/51,940	**0.64%**

*Data used with permission from American Association of Blood Banks (AABB) 2002 Annual Report.

Summary of STR Mutations

Mutations impact paternity testing and missing persons investigations but not forensic direct evidence-suspect matches…

- Mutations happen and need to be considered
- Usually 1 in ~1000 meioses
- Paternal normally higher than maternal
- VWA, FGA, and D18S51 have highest levels
- TH01, TPOX, and D16S539 have lowest levels


STRBase
Short Tandem Repeat DNA Internet Database
http://www.cstl.nist.gov/biotech/strbase
General Information
•Intro to STRs
(downloadable PowerPoint)
•STR Fact Sheets
•Sequence Information
•Multiplex STR Kits
•Variant Allele Reports
•Training Slides
Forensic Interest Data
•FBI CODIS Core Loci
•DAB Standards
•NIST SRMs 2391
•Published PCR Primers
•Y-Chromosome STRs
•Population Data
•Validation Studies
•miniSTRs
Supplemental Info
•Reference List
>2500
•Technology Review
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•DNA Quantitation
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New information is added regularly…